

## LGM Grand Rounds – September 22, 2022

**Objective:** At the conclusion of this presentation the attendees should be able to:

1. Describe CTCL and difficulty in the initial diagnosis.
2. Review T cell receptor (TCR) rearrangement in the context of clonality.
3. Compare the diagnostic modalities for CTCL.



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#### Disclosures:

**Course Chair:** Neil Anderson, MD: Speakers Bureau/Honoraria: Alere, Biomerieux; Consulting/Advisory Committee: Diasorin Molecular

**Planning Committee:** Nicole Tarlton: Nothing to disclose

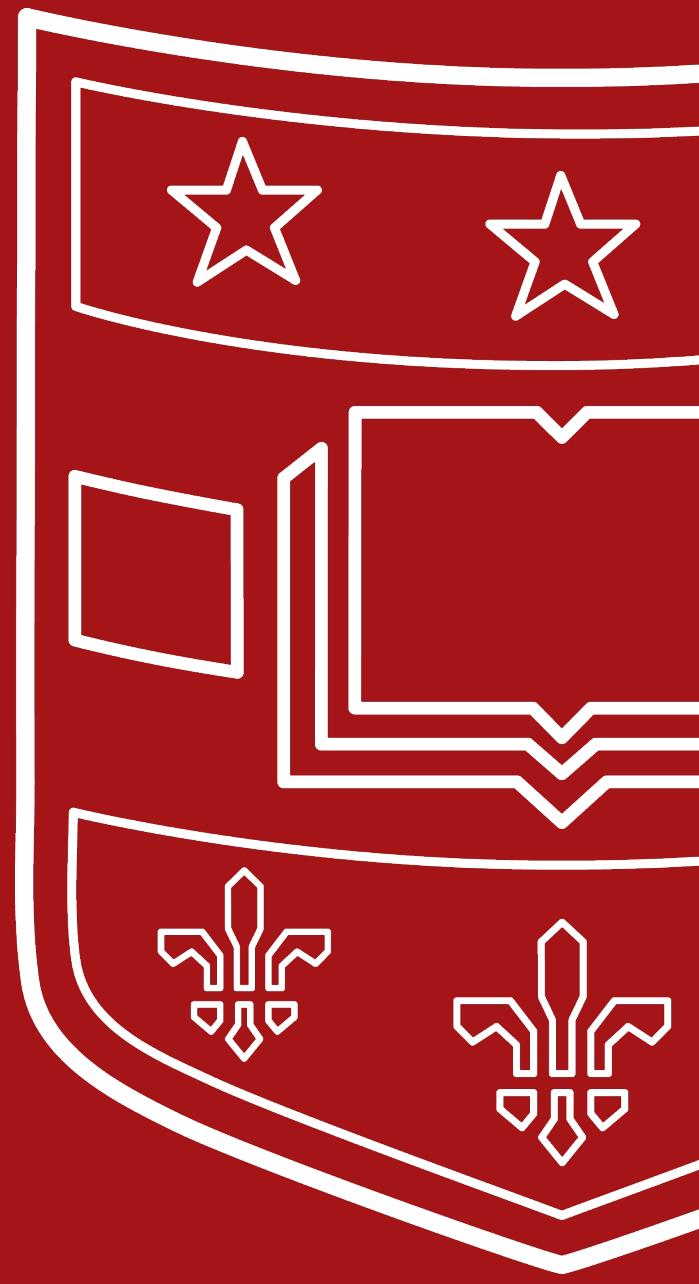
**Session Speaker:** Nick Borcherding, MD, PhD



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# The role of T cell receptor sequencing in the diagnosis and management of Cutaneous T Cell Lymphoma (CTCL)

Nick Borcherding  
CP-PSTP Resident, PGY-3  
LGM Grand Rounds, 9/22/2022





# Disclosures

- I have no conflicts of interest related to the content of this presentation



# Clinical Summary

76 YO male

- Hx diffuse large B-cell lymphoma in the setting of follicular lymphoma
- Rash that began ~ 1 month ago
  - Across torso with distinct raised patches
  - Rash is very pruritic
  - Topical hydrocortisone and anti-histamines have not been effective





# Clinical Course

- January 2014: Diagnosed as hypersensitivity reaction, treated conservatively
- May 2014: Seen at Mayo, skin biopsy taken and diagnosed with Pityriasis rubra pilaris
- November 2015: Secondary biopsy taken
  - Lichenoid infiltrate with nuclear contour irregularities, increased CD4 to CD8 ratio
- December 2015:
  - Flow cytometry performed showing increased CD4 to CD8 ratio

OSH Diagnosed as **Cutaneous T Cell Lymphoma (CTCL) 2016**

- Started on Targretin (Bexarotene), a modified Vitamin A derivative



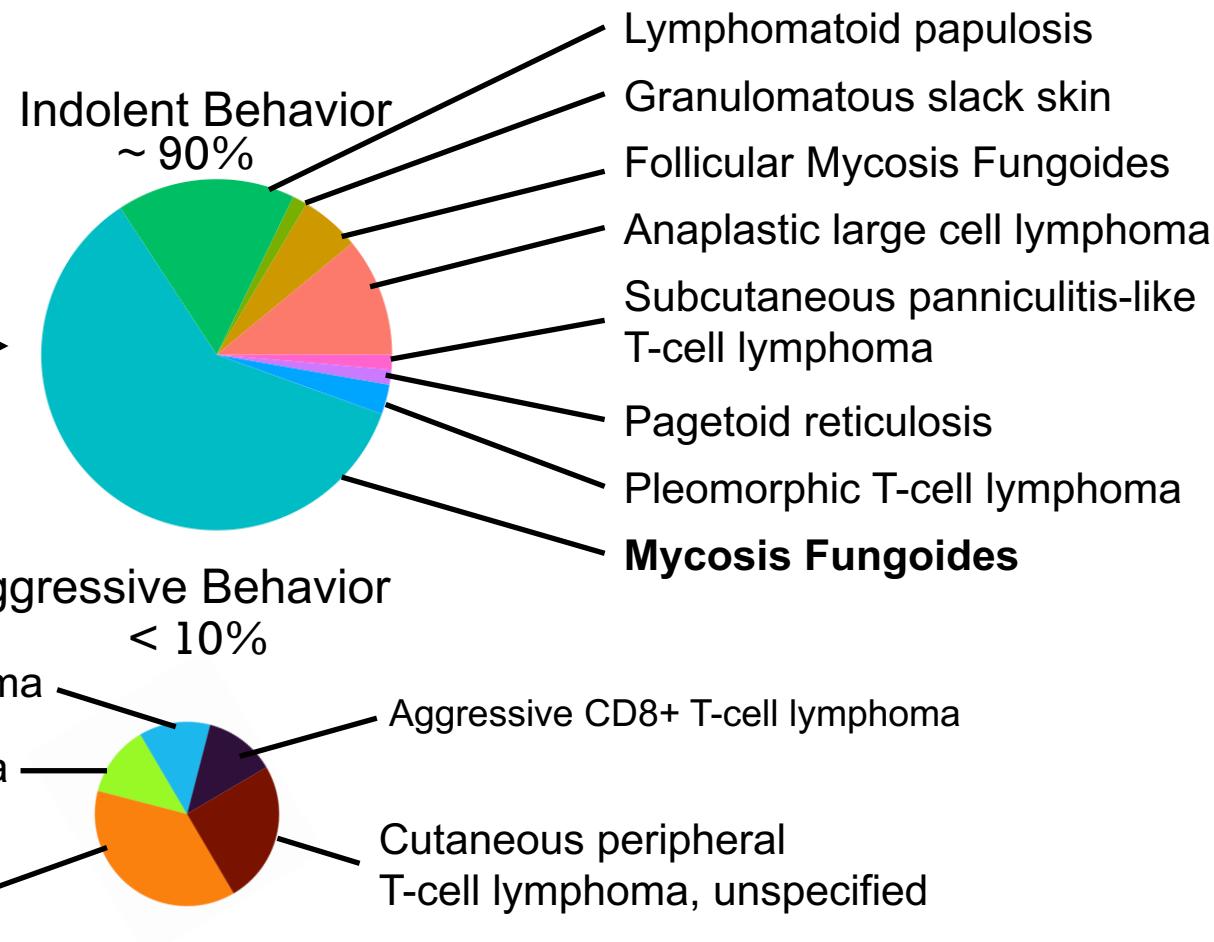
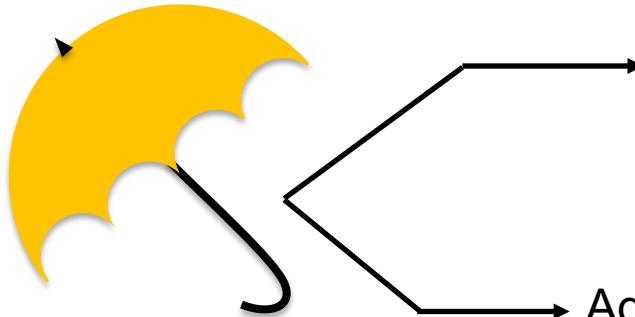
# Learning objectives

- Describe CTCL and difficulty in the initial diagnosis.
- Review T cell receptor (TCR) rearrangement in the context of clonality.
- Compare the diagnostic modalities for CTCL.



# What is cutaneous T cell lymphoma (CTCL) ?

Rare malignancy involving mature, skin-homing T cells



5 year survival  
>75%

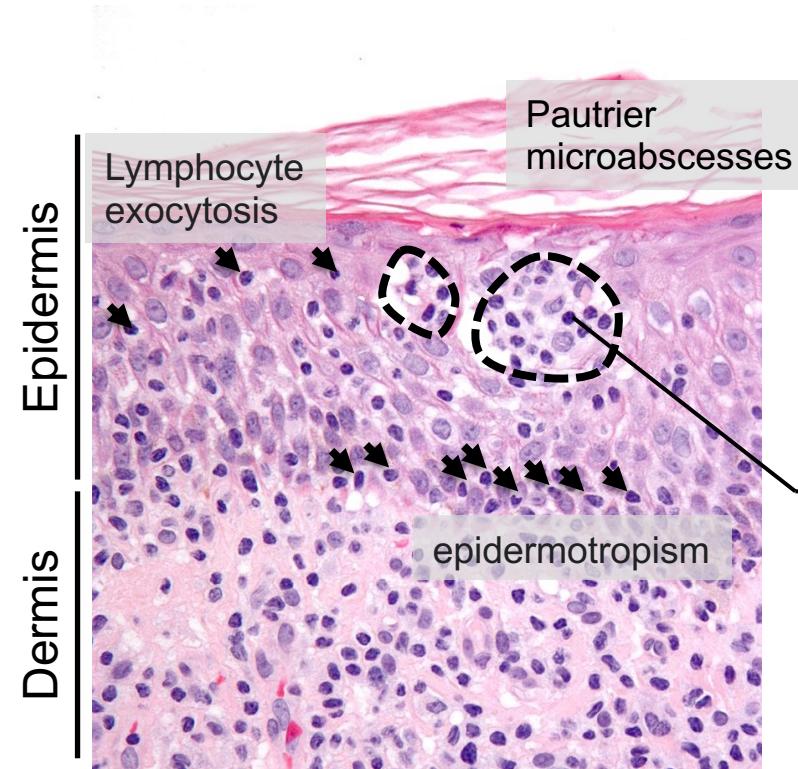
↓  
1/5 of patients  
progressive disease

5 year survival  
<20%

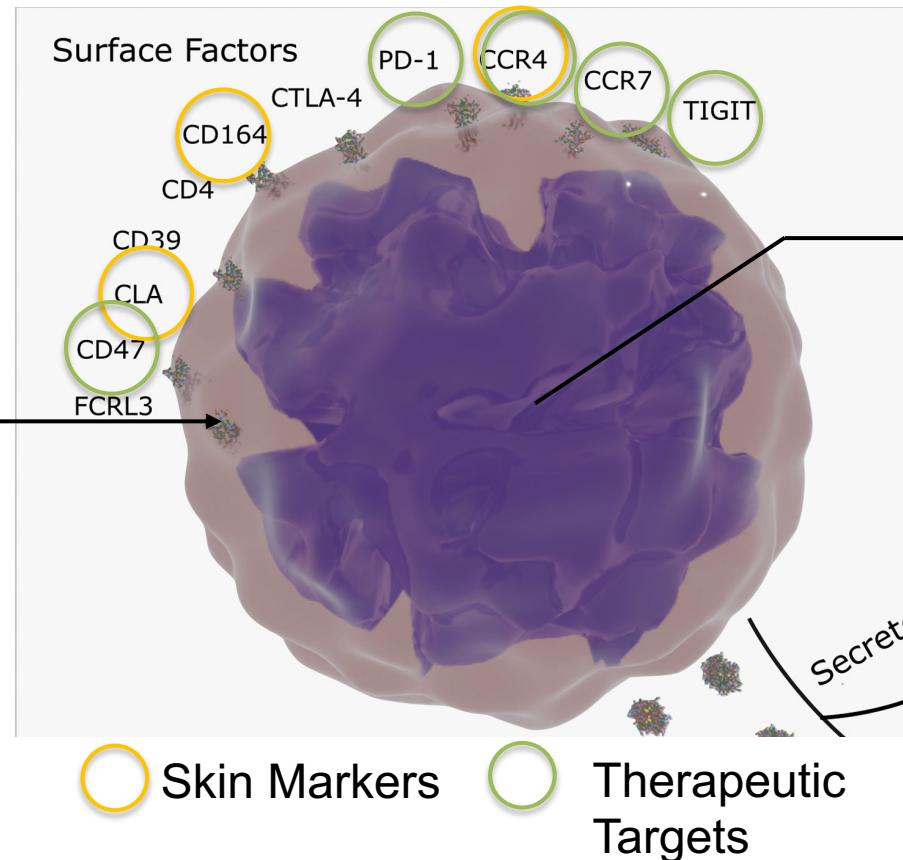
**Incidence:** 1 in 100,000  
**Prevalence:** 1 in 10,000  
**5-Year BJC Prevalence:** 1,159



# What is cutaneous T cell lymphoma (CTCL) ?



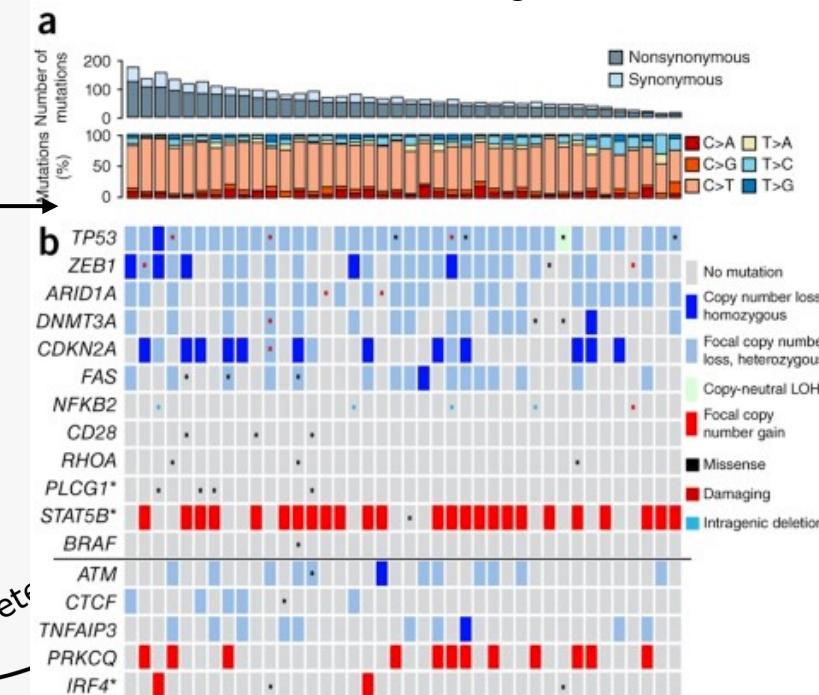
Skin-homing CD4+ T cells



○ Skin Markers

○ Therapeutic Targets

Disease of Copy Number Alteration  
92% of “driving” alterations



Median time to diagnosis for early disease: **6 years**

What makes CTCL difficult to diagnose?

Choi et al. Nature Genetics 2015  
Durgin et al JAAD 2020

# Diverse cutaneous presentations of CTCL leads to confusion in diagnosis



Images from Dr. Vincent Liu, University of Iowa



# Which of the following is an example of CTCL?

Mycosis Fungoides



Mycosis Fungoides



Mycosis Fungoides



Sezary Syndrome



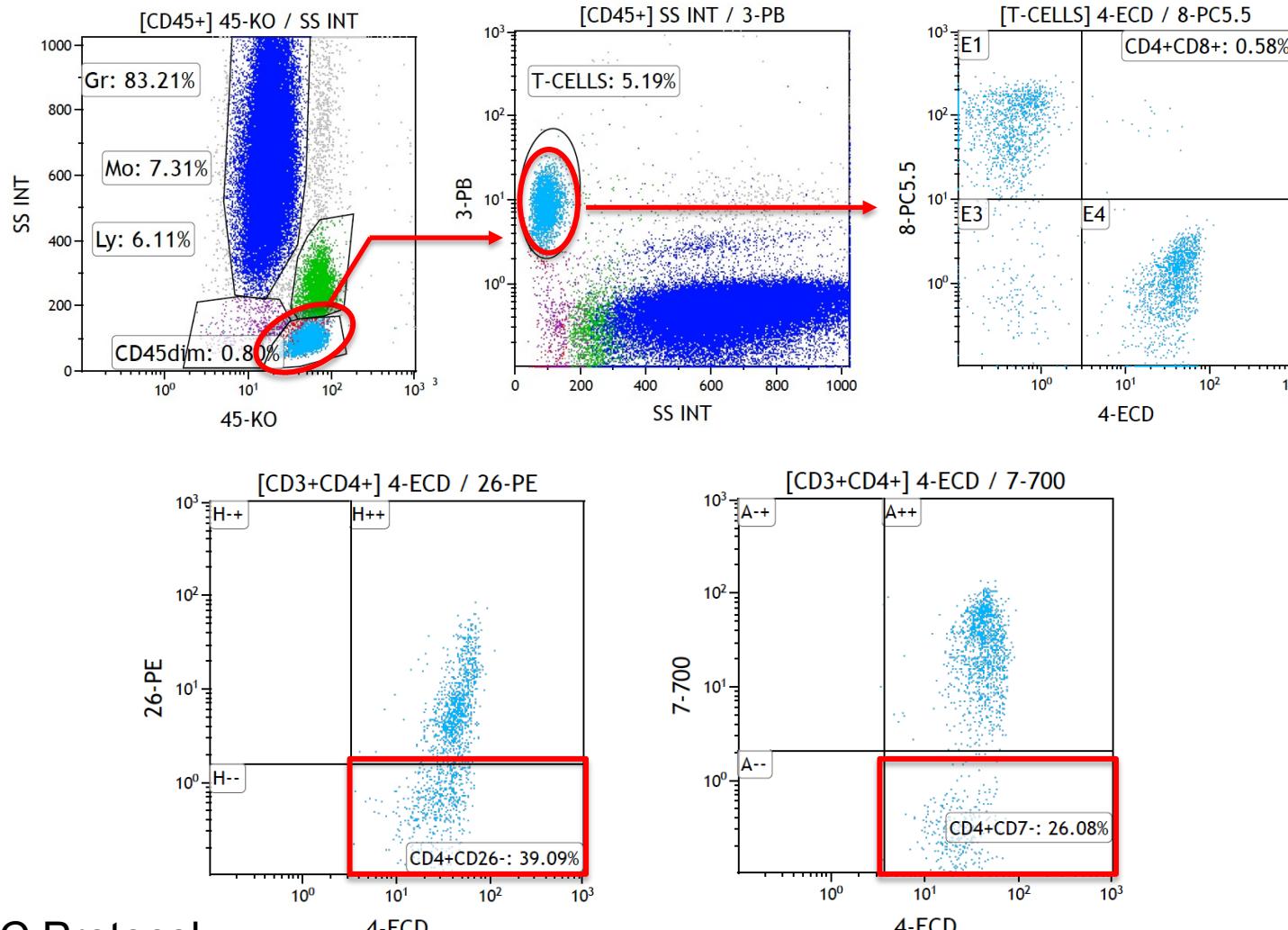
Skin manifestations can be mistaken for number of conditions:

- Eczema
- Psoriasis
- Dermatitis
- Dry Skin
- Pityriasis rubra pilaris

Histology is supportive but not diagnostic

Reliance of flow cytometry for diagnosis

# Flow cytometry: Mainstay of Diagnosis and Therapeutic Monitoring



[T-CELLS] % Gated	5.2
[T-CELLS] Number	3,102
CD4:CD8 RATIO	1.42
T-Cell Content:	
[CD3+CD4+] % Gated	56
[CD3+CD8+] % Gated	39.5
[CD4+CD8+] % Gated	0.6
[CD4+CD7-] % Gated	26.1
[CD4+CD26-] % Gated	39.1
[CD4+CD7-CD26-] % Gated	22.8
[CD52+] % Gated	97.4
[CD30+] % Gated	0.12
[CD3% OF LYMPHS] % Gated	84.2

# Diagnosing CTCL depends on Flow and TCR rearrangement



## ISCL-USCLC-EORTC 2021

Clone identified by TCR gene rearrangement +  $\geq 1$  of the following:

- Absolute count of  $\geq 1000/\text{ml}$  of:
  - CD4+/CD26-
  - CD4+/CD7-
  - Other aberrant population by flow

## WHO 2022

Clone identified by TCR gene rearrangement +  $\geq 1$  of the following:

- Absolute Sezary count  $> 1\text{k}/\mu\text{L}$
- CD4/CD8 ratio  $\geq 10$
- CD4+/CD7- cells  $\geq 30\%$
- CD4+/CD26- cells  $\geq 40\%$

Performance of the diagnostic criteria dependent on the disease process and tissue

### SS compared to Erythrodermic Inflammatory Dermatosis

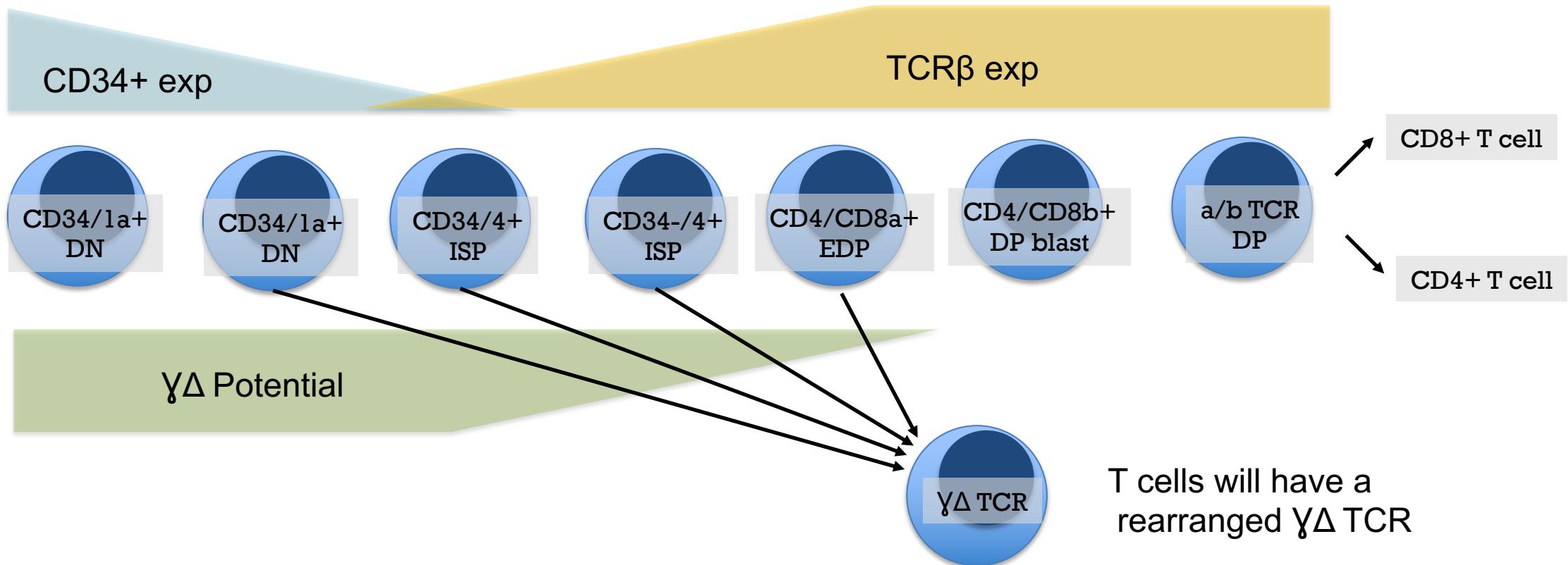
Sensitivity: **54%**

Specificity 100%

Sensitivity: 86%

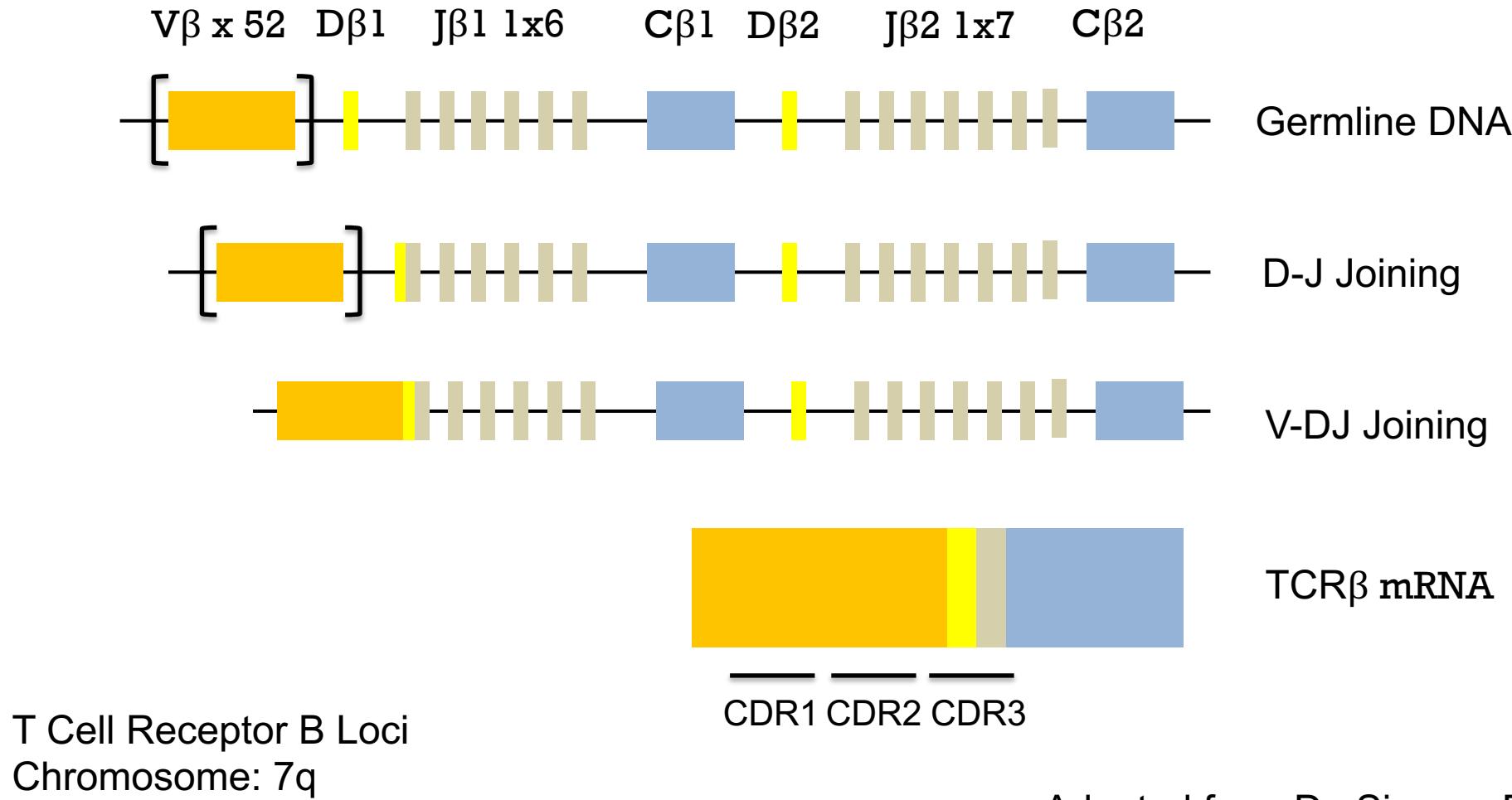
Specificity **47%**

# Thymus: T cell boot camp with clonal rearrangements





# VDJ Recombination generates unique TCRs



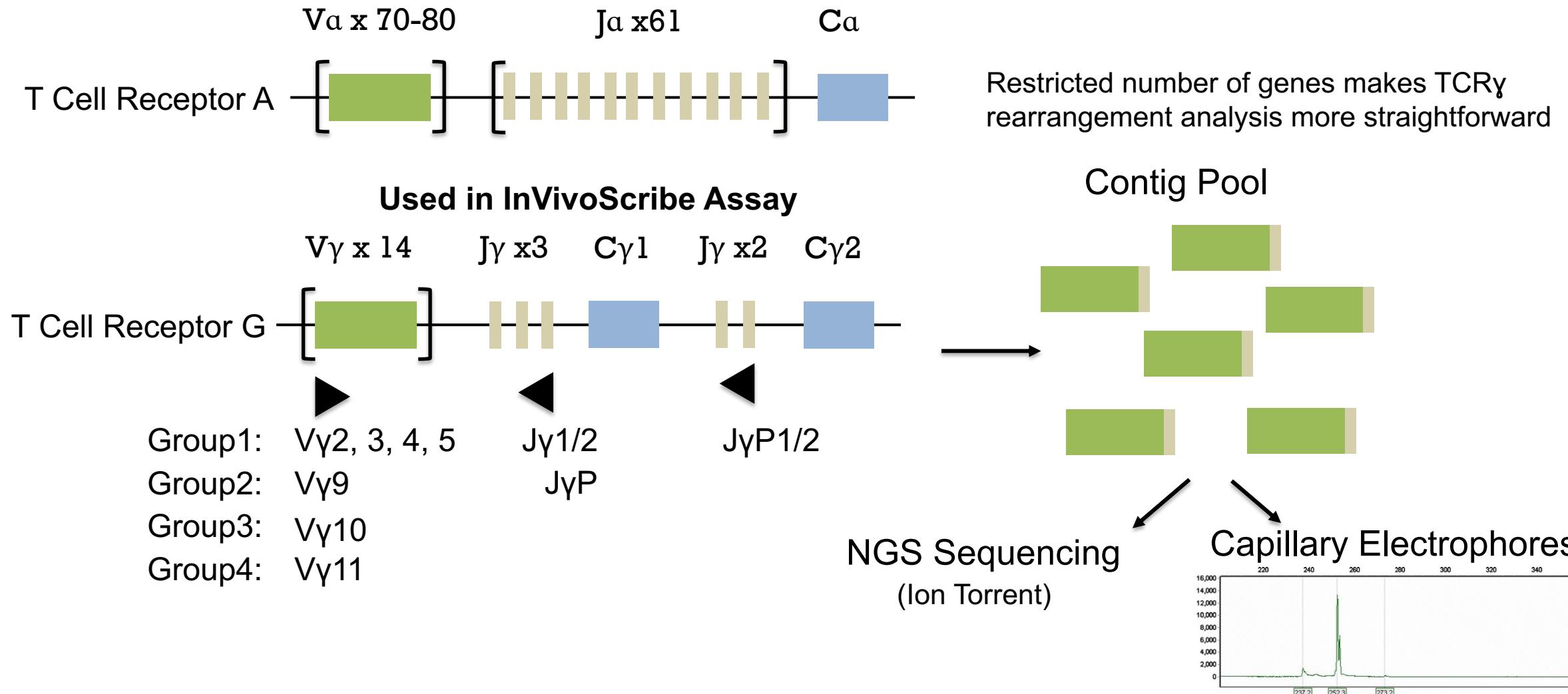
Gene Usage +  
Insertions +  
Deletions

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**10<sup>14</sup> unique  
sequences**



# TCR sequencing using the InVivoScribe Assay





# TCRG Sequencing-Based Clinical Report

## T Cell Receptor Gamma (TCRG), T cell clonality

Reported: 7/21/2022

Fresh abdominal tissue collected on 07/15/2022.

**Result: Clonal**

TRG Clonal Rearrangements	TRG V-genes	TRG J genes	% Total Reads
Rearrangement 1	Vg10	Jg1/2	10.85
Rearrangement 2	Vg4	Jg1/2	5.36

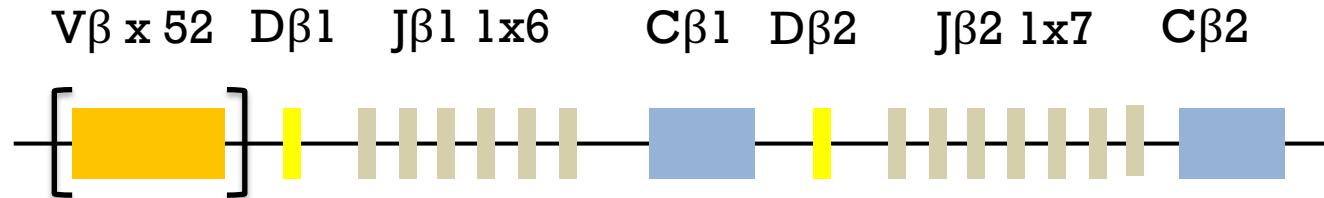
### **Interpretation**

Clonal *TRG* gene rearrangement(s) detected. The *TRG* Variable (V) and Joining (J) gene segments and percentage of total sequencing reads are reported in the table above. The cutoff for detection of a clonal rearrangement is  $\geq 2.5\%$  of total sequencing reads. These results are consistent with a clonal proliferation of T lymphoid cells, which may be indicative of a T lymphoid malignancy, but may be present in non-neoplastic inflammatory processes. Clinical correlation is recommended.

Performance dependent on sampling and type of tissue.



# PCR-based TCR $\beta$ assay compared to TCR $\gamma$



- |        |                        |                         |                         |
|--------|------------------------|-------------------------|-------------------------|
| Tube A | ► 23 V $\beta$ primers | ◀ 6 J $\beta$ 1 primers | ◀ 3 J $\beta$ 2 primers |
| Tube B | ► 23 V $\beta$ primers |                         | ◀ 4 J $\beta$ 2 primers |
| Tube C |                        | ► 2 D $\beta$ primers   | ◀ 13 J $\beta$ primers  |

- Compared to TCR $\gamma$
- **74 primers** compared to primers
  - Sequence **only on Illumina platforms**
  - TCR $\beta$  is the standard for reported sequence in TCR biology

**TCR $\beta$**

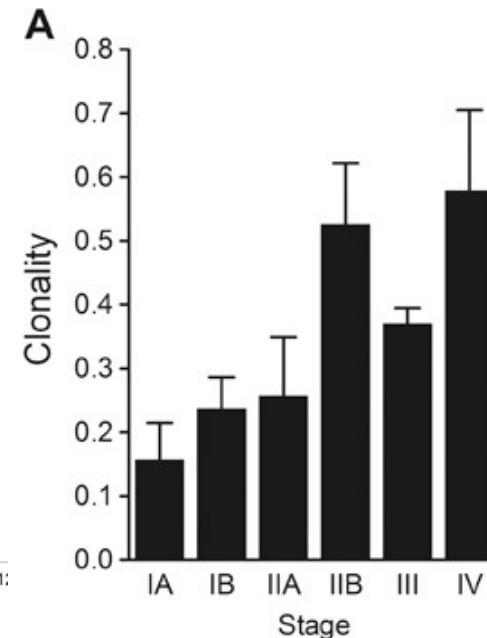
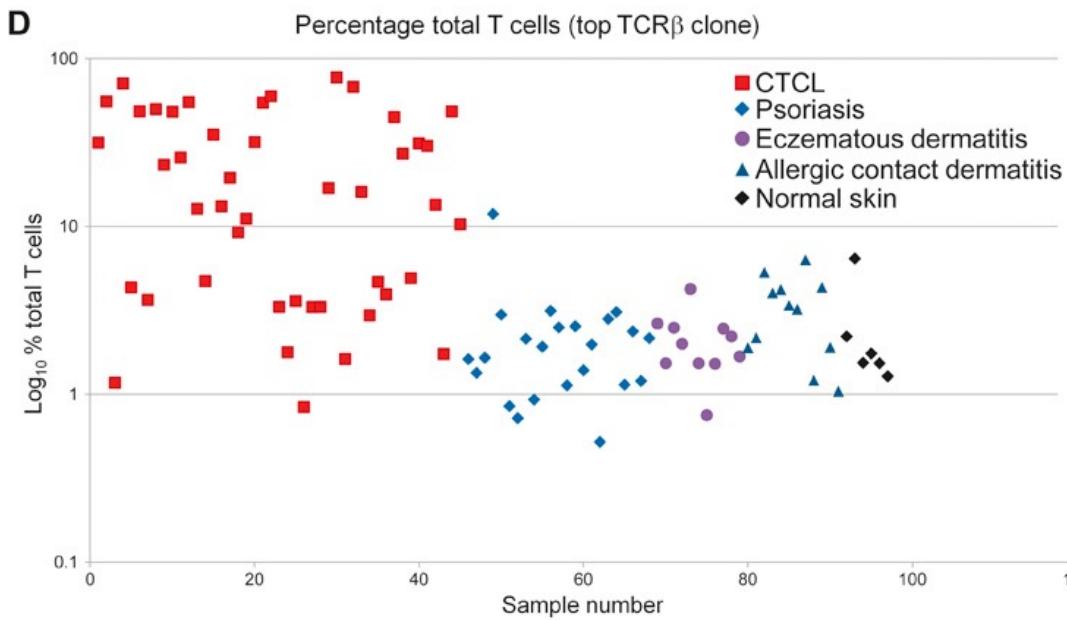
	Monoclonal	Polyclonal	Oligoclonal	Total
Monoclonal	43	22	1	66
Polyclonal	21	107	0	128
Oligoclonal	2	0	6	8
Total	66	129	7	202

Concordance Rate:  
77.2%

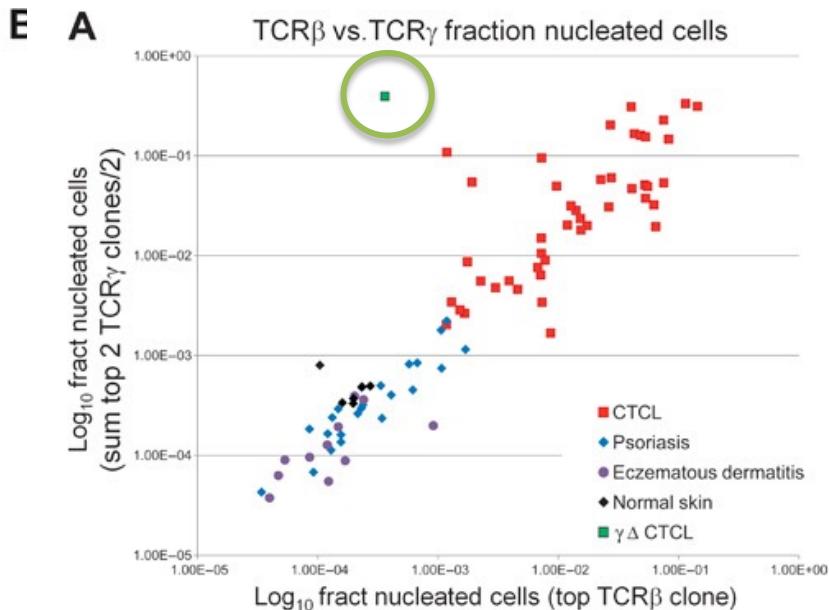


# Comparing TCR $\beta$ vs TCR $\gamma$ sequencing in CTCL

Clonality clearly defines CTCL and associated with advanced stage



Correlation of TCR $\beta$  to TCR $\gamma$



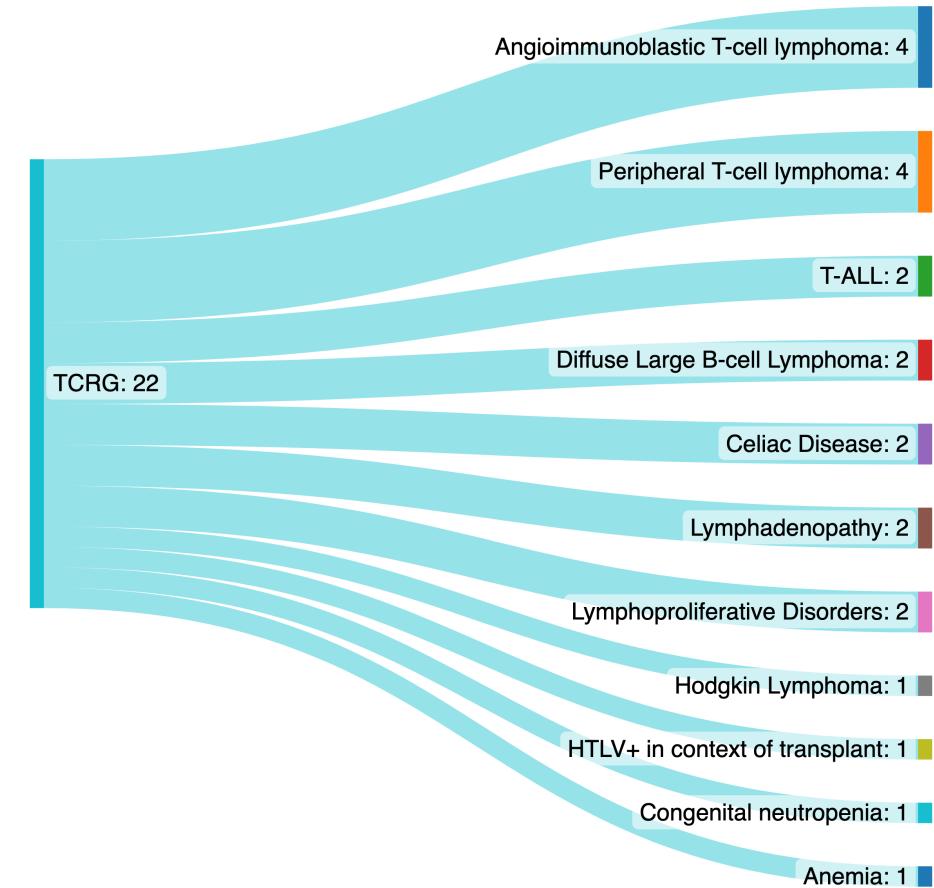
# TCRγ sequencing from the Molecular Diagnostic Lab



**Goal:** Compare Use of TCRγ Sequencing to Flow Cytometry for CTCL

From January 2019 to December 2020

- 38 TCRγ InVivoScribe Assays were performed
- 22 Unique Patients



**Unfortunately No Use of TCRγ Sequencing in CTCL**

- Referral center nature of BJC?
- TCRγ sequencing is not routinely used for disease monitoring



Lets Check Some Literature!



# Towards early CTCL detection and diagnosis

Research

JAMA Dermatology | Original Investigation

## T-Cell Receptor Gene Rearrangement Clonality, Flow Cytometry Status, and Associated Outcomes in Early-Stage Cutaneous T-Cell Lymphoma

Jennifer A. Marks, MD; Jeffrey M. Switchenko, PhD; Dylan J. Martini, MD; Erica S. Tarabadkar, MD;  
Mohammad K. Khan, MD, PhD; Mary Jo Lechowicz, MD; Pamela B. Allen, MD

**Objective:** To assess the association of **low-level blood** involvement by TCR clonality and flow cytometry with outcomes for patients with early-stage CTCL.

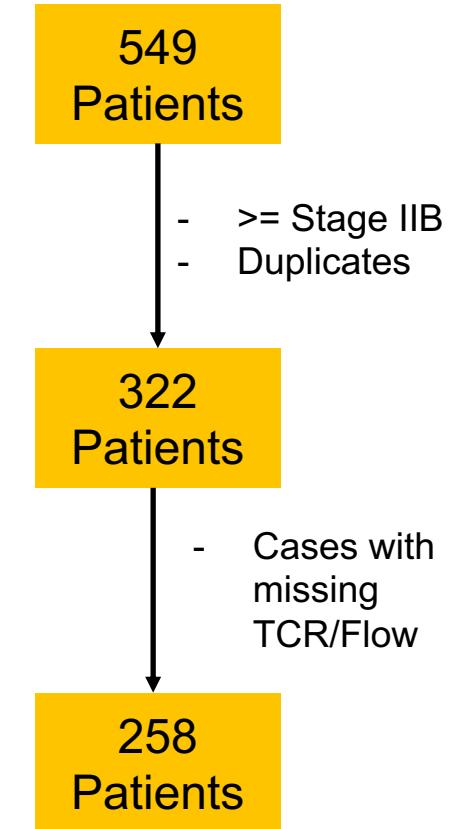
Patients identified from 1990-2018

### Flow Criteria for Inclusion

- CD4/CD8 ratio  $\geq 10$
- CD4+/CD7- cells  $\geq 30\%$
- CD4+/CD26- cells  $\geq 40\%$

### TCR Rearrangement Inclusion

- InVivoScribe designated clonal





# Performance of Flow vs TCR rearrangement in early disease diagnosis

Flow Cytometry

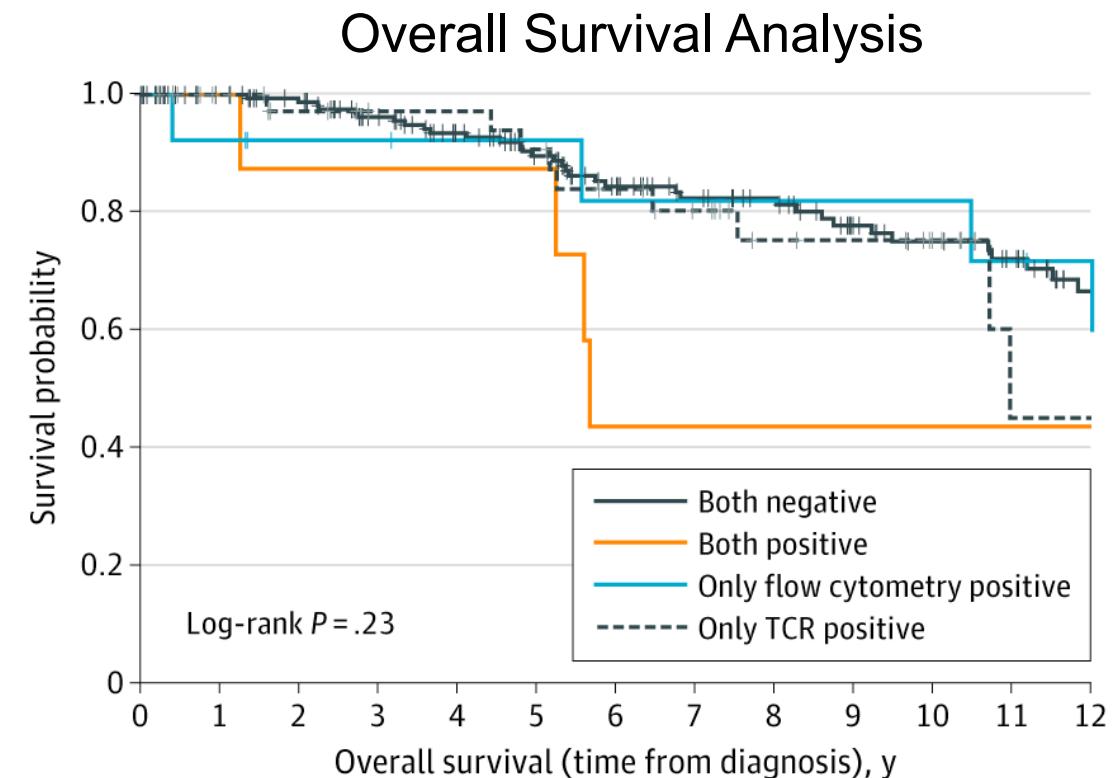
N = 26 of 295  
Sensitivity: 8.8%

TCR  
Rearrangement

N = 55 of 263  
Sensitivity: 20.9%

Overall  
Performance

**Both positive:** 10 of 258  
**1 Positive:** 56 of 258  
**Both Negative:** 192



Diagnosis to TCR+ > 6 months: HR 0.58 (P < 0.001)



# Take away message from publication

- **Peripheral blood TCR sequencing is more sensitive than flow cytometry in early disease detection.**
- Delay in detection of clonal TCR sequence may serve as a **prognostic marker for disease.**

## Limitations

- Missing assessments:
  - TCR clonality: **18%**
  - Flow cytometry: **8.4%**
- > 6 months between diagnosis and assessment associated with bias in disease staging
  - TCR clonality: **40.2%**
  - Flow cytometry: **38.5%**

# Comparing lesion vs peripheral blood sampling for diagnosis in CTCL



## **Utility of flow cytometry and gene rearrangement analysis in tissue and blood of patients with suspected cutaneous T-cell lymphoma**

JULIE D. GIBBS<sup>1\*</sup>, SOPHIA MA<sup>2\*</sup>, ANNA KIM<sup>3</sup>, LUCIA SEMINARIO-VIDAL<sup>3</sup>, LUBOMIR SOKOL<sup>4</sup>, HAILING ZHANG<sup>5</sup>, XIAOHUI ZHANG<sup>5</sup>, ELIZABETH SAGATYS<sup>5</sup>, PEI-LING CHEN<sup>5</sup> and JANE L. MESSINA<sup>6</sup>

**Objective:** Compare Flow Cytometry to TCR Rearrangement via PCR and high throughput sequencing.

Mixed cohort of confirmed CTCL and suspected CTCL

No specifics in methods on the confirmation of suspected CTCL

### Flow Criteria

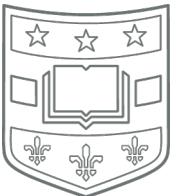
T cell population with aberrant loss of CD7, CD26 or other pan-T-antigens

### TCR PCR

Single peak > 2.5x larger than adjacent peaks

### TCR HTS

Single sequences > 5% of total sequences



# Lesion-specific performance of CTCL assessment

Missing TCR-PCR: 13.3%  
Missing TCR-HTS: 56.6%

	Patch	Plaque	Tumor	Erythroderma	Overall	
TCR-PCR	Sensitivity	55%	82%	0%	0%	61%
	Specificity	66%	50%	100%	50%	64%
TCR-HTS	Sensitivity	25%	82%	0%	0%	60%
	Specificity	66%	50%	100%	50%	87%
Flow Cytometry	Sensitivity	55%	75%	0%	50%	66%
	Specificity	100%	100%	100%	100%	100%

# Peripheral Blood-specific performance of CTCL assessment



Missing TCR-PCR: 5.6%  
Missing TCR-HTS: 69.8%

	Patch	Plaque	Tumor	Erythroderma	Overall
TCR-PCR	Sensitivity	38%	36%	0%	75%
	Specificity	71%	57%	0%	100%
TCR-HTS	Sensitivity	0%	50%	0%	NA
	Specificity	100%	100%	0%	NA
Flow Cytometry	Sensitivity	23%	0%	0%	50%
	Specificity	85%	83%	100%	75%

# Take away message from lesion vs blood comparison



- Samples from lesions had higher rates of positive testing
- TCR Evaluation:
  - TCR-PCR vs TCR-HTS has similar sensitivities, but **TCR-HTS had greater specificity**
  - TCR-HTS had higher rates of **adequate sampling compared to Flow or TCR-PCR**

## Limitations

- **Missing data (>50% for TCR-HTS)** makes performance and generalizability difficult
- No clear explanation of prospective/retrospective cohort combined.

# Clinical Case Revisited

Inconclusive

Patient re-establishing care at BJC May 2022 with new rash

- **Flow cytometry:** skin lesion shows population of CD4+ CD7- cells < 30 %
- **Histology:** spongiotic dermatitis at dermoepidermal junction comprised of CD4+ and CD8+ T cells

TCR $\gamma$   
sequencing

Date Reported	Accession Number	Specimen Number	Specimen Type	VJ Genes	% Total Reads
7/21/2022	BJM22-184		Tissue R. Thigh	Vg10	Jg1/2 25.24
				Vg4	Jg1/2 10.34

Patient seen at BJC in 2014/2015 for rash issue and TCR $\gamma$  rearrangement was performed using capillary electrophoresis

Date Reported	Accession Number	Specimen Number	Specimen Type	VJ Genes	% Total Reads
12/24/2014 (CE)		D14-13351	Mid-Back	Vg10	Jg1/2 23.90
7/29/2022 (NGS)				Vg4	Jg1/2 15.51
1/4/2015 (CE)		D15-15320 B1	L. Lower	Vg10	Jg1/2 38.89
7/29/2022 (NGS)			Back	Vg4	Jg1/2 23.91

Dx of CTCL  
reccurence



Single Dose  
Mogamulizumab  
(anti-CCR4)



# Conclusions

- TCR sequencing is **underutilized for diagnosis or monitoring of CTCL at BJC**
- **Combined TCR $\gamma$ /TCR $\beta$  assay may offer improved detection of clonal TCRs**
  - No direct comparison exist in literature with added TCR $\beta$  in CTCL
  - InVivoScribe TRB assay does runs on Illumina
- TCR sequencing **may offer longitudinal resolution** for assessment of recurrence/continued disease.



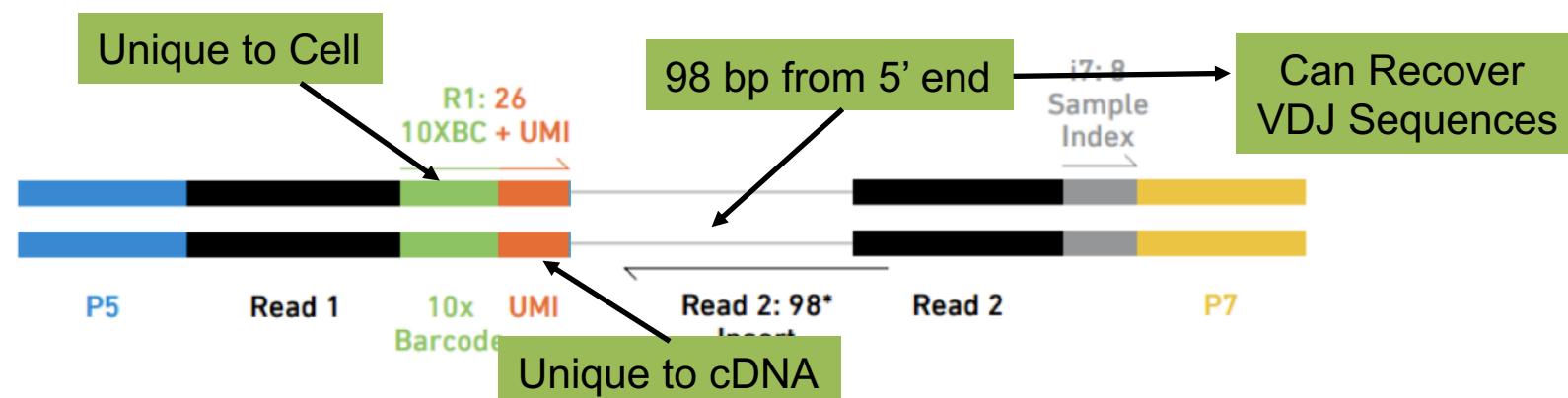
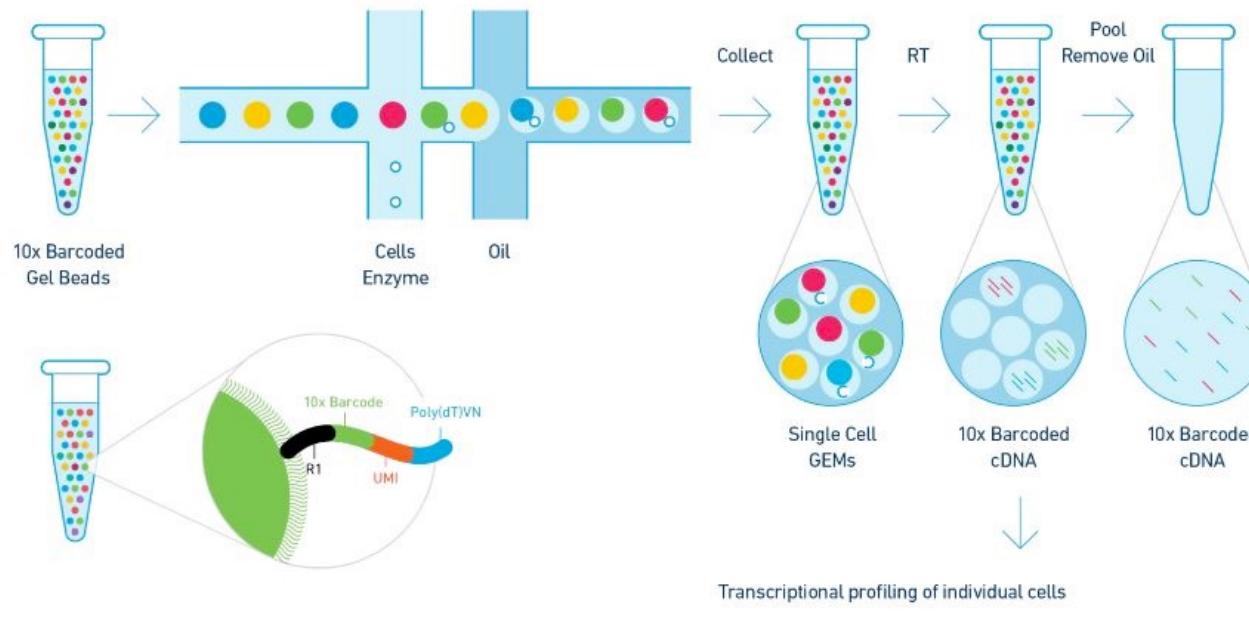
# Intriguing Questions

Is there a different technology that could combine the approaches for CTCL diagnosis?

Can TCR sequences be used beyond a measure for clonality?



# Singling out use of single-cell data



## Advantages

- T cell subtype information
- Able to simultaneous get RNA and TCR

## Disadvantages

- Expensive
- Requires computational infrastructure
- Drop-Out effect



# CTCL Single-Cell RNA/TCR cohort

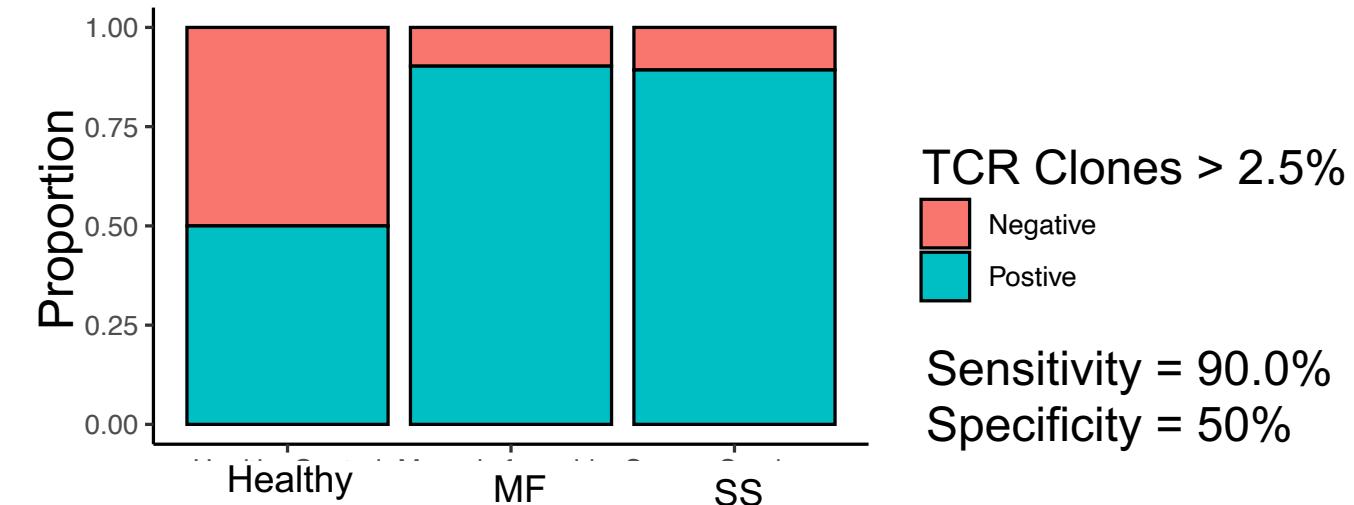
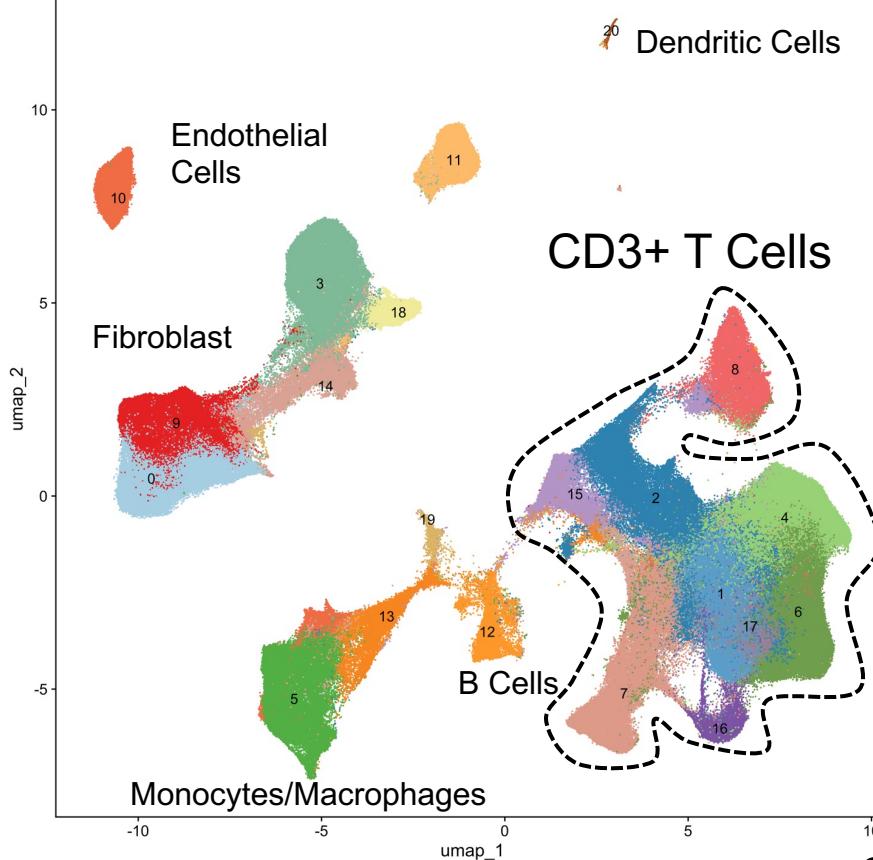
126 Sequencing Runs

77 Patients

733,895 Cells

Samples from skin and peripheral blood

3 Tb data → 20 Gb item



Single-Cell RNA quantification is likely insufficient for diagnostic purposes

TCR Clones > 2.5%  
Negative  
Positive  
Sensitivity = 90.0%  
Specificity = 50%



# Single-cell protein level quantification of MF/SS

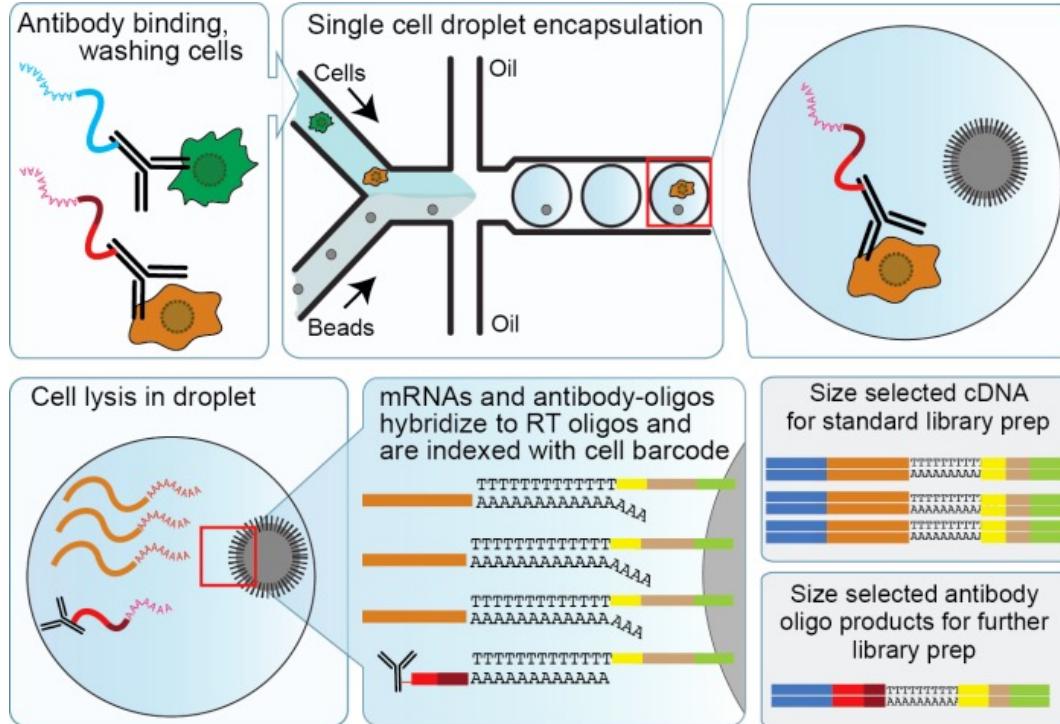
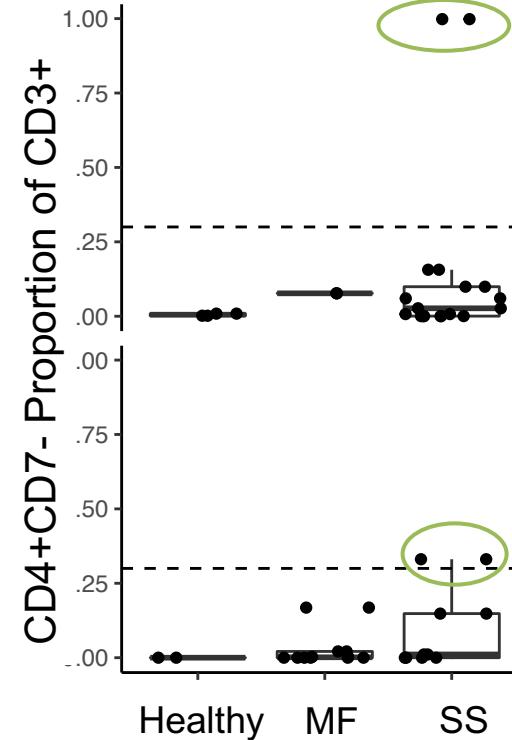
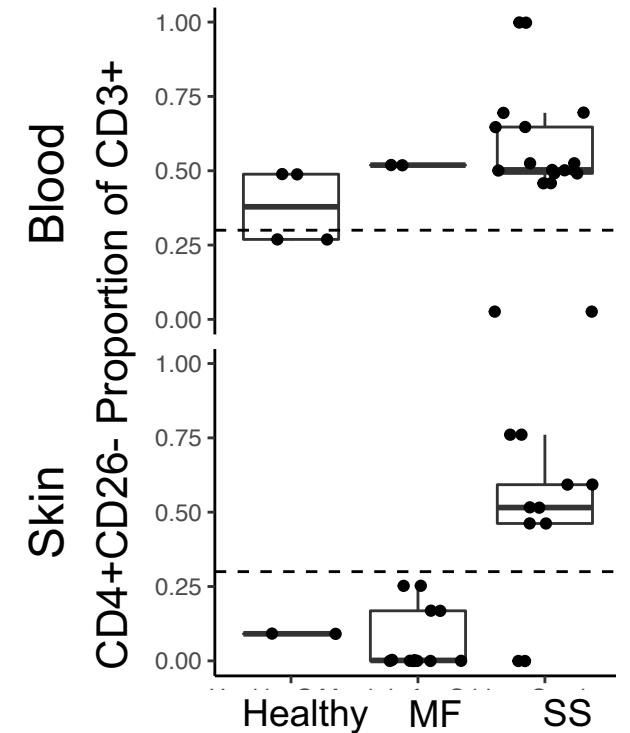


Image from NYGC Technology Innovation Lab



Sensitivity = 9.5%  
Specificity = 100%



Sensitivity = 61.9%  
Specificity = 67%

Possible use of single-cell protein-level + TCR quantification



# Can TCR sequences be used beyond a measure for clonality?

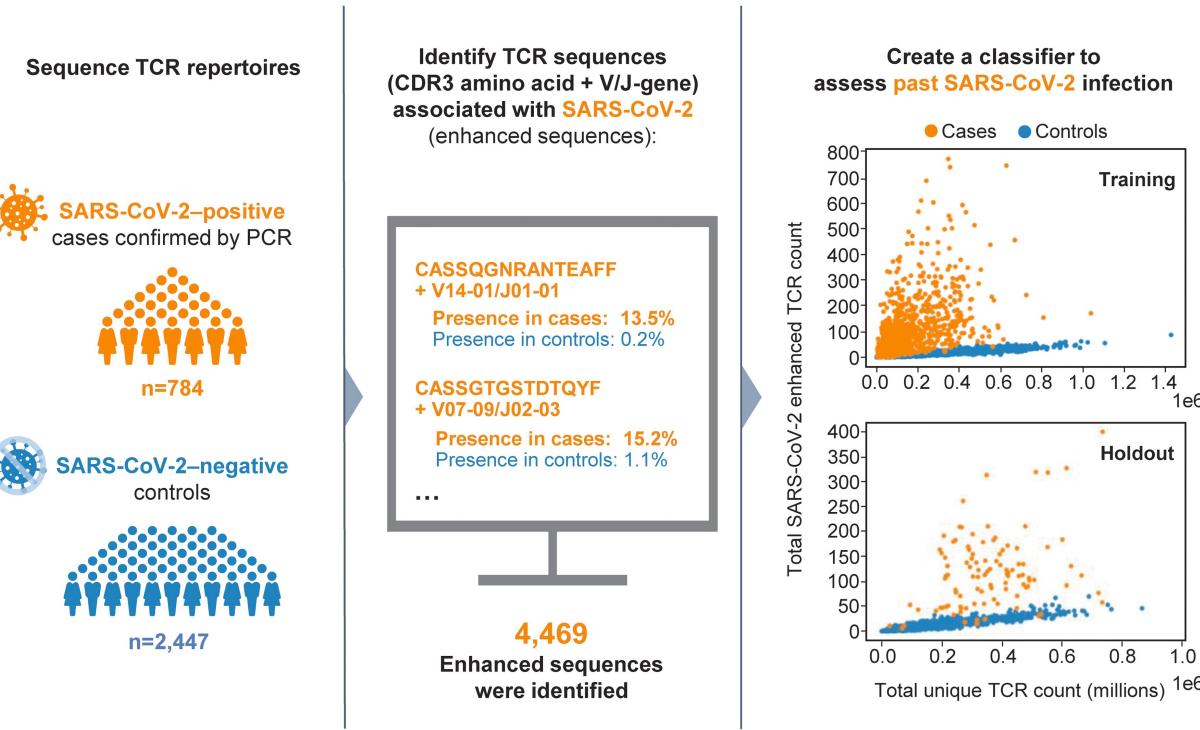
From Adaptive Biotechnologies



## T-Detect

First T-cell assay for detection of recent or past COVID-19

Days Since Symptom Onset	Samples, n	T-Detect COVID PPA	Abbott Architect SARS-CoV-2	Roche Elecsys Anti-SARS-CoV-2
0–7	13	53.8 (25.1–80.8)	15.4 (1.9–45.4)	15.4 (1.9–45.4)
8–14	9	77.8 (40–97.2)	22.2 (2.8–60)	22.2 (2.8–60)
≥15	55	94.5 (84.9–98.9)	88 (75.7–95.5)	90.4 (79–96.8)



This technique may be applicable to CTCL as there is a limited TCR repertoire that may be reactive to *S. aureus*



# Learning objectives

- Describe CTCL and difficulty in the initial diagnosis.
- Review T cell receptor (TCR) rearrangement in the context of clonality.
- Compare the diagnostic modalities for CTCL.



# Acknowledgements

- Dr. Bijal Parikh
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- Dr. Vincent Lui (Ulowa)
- Dr. Ali Jabbari (Ulowa)

George Borcherding



Ava Borcherding

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**Planning Committee:** Nicole Tarlton: Nothing to disclose

**Session Speaker:** Nick Borcherding, MD, PhD



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